SUBSTITUTE SPECIFICATION

PROCESS FOR THE PREPARATION OF MALTED CEREALS

FIELD OF THE INVENTION

[0001] The present invention is related to an improved process for the preparation of maltedcereals, the improved malted cereals obtained and their use, especially in biotechnological processes for the preparation of beverages.

TECHNOLOGICAL BACKGROUND OF THE INVENTION

[0002] Cereals, such as barley, wheat, rye, corn, oats, rice, millet triticale, and sorghum, are used for the production of beverages. In most cases, they have been subjected to a malting process to take advantage of their increased enzymatic potential.

[0003] In traditional malting processes, the moisture content of cereals is raised either by immersion(s) and/or spraying(s), and the resulting high-moisture content cereal is allowed to germinate. After reaching the proper physiological condition, it is preferably submitted to (a) drying step(s). In what follows, the term steeping refers to the increase in moisture level, while the term germination is used in the way it is in plant physiology. The drying operations are referred to as kilning and the term malting involves all operations needed to convert barley (or other cereals) to barley malts (or other cereal malts).

[0004] The quality of the malt obtained is, to a large extent, determined by the presence of plant endogenous enzymes generated during the melting process. For instance, with cereals like barley used as a raw material for the malt production, the variety, the composition of the microbial flora and the environmental factors, such as agricultural practice, influence the quality of the malt. During cultivation and storage, cereals are contaminated with bacteria and fungi. In the malting plant, neither the air, the water, nor the equipment are sterile, and the conditions of humidity, pH and temperature favor the growth of the microbial populations. To improve the quality of malted cereals, such as barley, enzymes have been added to the malted cereal.

[0005] The variable cereal quality and the lack of means to make up for deficiencies during the malting process result in variability in malt quality and enzymatic activity. In many instances, this has to do with an imbalance of specific enzymatic potential and insufficient cell wall degradation. Apart from this, problems with microbial safety canoccur. As a consequence of the defects in malt, quality problems occur in the production of beer, such as a poor filtration of the wort.

STATE OF THE ART

[0006] During the malting of cereals, the microflora present on the cereals develops and the quality of the malt and beverages is influenced by the activity of the latter microorganisms.

[0007] In analogy with other biotechnological processes, there have been attempts to optimize malt quality aspects by the addition of starter cultures during the malting process (Boivin, P. & Malanda, M., Influence of Starter Cultures in Malting on the Microflora Development and Malt Quality, EBC, Proceedings of the 24th Congress, pp. 95-102 (1993); Haikara, A. et al., Lactic Starter Cultures in Malting - A Novel Solution to Gushing Problems, European Brewery Convention, Proceedings of the 24th Congress, pp. 163-172 (1993)).

[0008] Addition of spores of Geotrichum candidum to the steeping water results in the inhibition of the development of undesirable microorganisms and in a decrease of the filtration time of wort made of the obtained malt. Treatment with Geotrichum candidum also inhibits the formation of mycotoxins by Fusarium spp.

[0009] The influence of Lactobacillus plantarum and Pediococcus pentosaceus has been tested on the microflora during malting, and it has been found that these cultures act as natural preservations as they restrict the growth of Fusarium and prevent gushing.

[0010] The international patent application WO 94/29430 describes a process for improving the properties of malted cereals wherein starter cultures which comprise moulds, yeasts or bacteria

are added prior and/or during malting of said cereals.

[0011] The preferred bacteria used are lactic acid producing bacteria, such as various Lactobacilli, e.g., Lactobacilluscasei, Lactobacillus casei var rhamnosus, Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis, and bacteria of the genus Pediococcus, e.g., Pediococcus acidilactici.

[0012] Preferred moulds are moulds of the genus Aspergillus and Geotrichum, like Geotrichum candidum.

[0013] The international patent application WO94/16053 describes a process for treating cereals for inhibiting growth of unwanted microbial species by inoculating the cereals during the germination process with a lactic acid bacteria preparation or a preparation produced by lactic acid bacteria. The preferred bacteria are lactic acid bacteria belonging to genus Lactococcus, Leuconostoc, Pediococcus or Lactobacillus.

[0014] The British patent application GB-1211779 provides a method for the automatic control and regulation of a malting process. It enables one to determine the parameters necessary for a successful automatically controlled and regulated malting process.

[0015] In the proceedings of the European Brewery Convention, volume 16, 1977, pages 245 to 254, the influence of some fungi on malt quality is described, more specifically, contamination of barley malt with fungi which has led to gushing and other qualitative changes in the beer.

[0016] The German patent application DE-3028360 discloses a method to make malt out of corn.

[0017] However, malt prepared according to the present invention is of better quality than that prepared according to WO 94/29430. This is exemplified by higher β-glucanase and xylanase activities, lower glucan contents in malt and wort and improved European Brewery Convention analytical data.

AIMS OF THE INVENTION

[0018] The present invention aims to provide an improved preparation process for malted cereals and improved malted cereals.

[0019] A main aim of the invention is to provide an improved preparation process for malted cereals and improved malted cereals in terms of brewing performances, especially malted cereals having an improved quality in terms of enzymatic potential and microbial safety.

[0020] Another aim is to provide a process and improved malted cereals which vary less in quality with the raw material used.

[0021] A further aim of the invention is to obtain malted cereals which improve the biotechnological production process of beverages and may improve the properties of the said obtained beverages.

SUMMARY OF THE INVENTION

[0022] The present invention is directed to a process for the preparation of a malted cereal, the malted cereal which is the product of the process of the invention, and a combination of wetted or moistened cereal and activated spores which, when held for a sufficient time and temperature, provide a malted cereal product of enhanced enzymatic activity. The product of the invention has enhanced enzymatic activity of at least one enzyme, such as β-glucanase, xylanase, amylase, naturally occurring enzymes, and/or protease activity, over malted cereal products which are similarly prepared with wetted cereal products with or without microorganisms.

[0023] The process of the invention utilizes activated spores from microorganisms such as bacteria or moulds. The process generally comprises combining water, the cereal and activated spores and holding the combination until a malted cereal of enhanced enzymatic activity is formed. Generally the combination is made by inoculating the moistened cereal with the activated spores, but the activated spores and cereal may be combined before or after the

moistening of the cereal. In the process of the invention, the combination of wetted cereal and activated spores has a concentration of activated spores, holding time and holding temperature which are effective for providing the malted cereal with an increase in enzymatic activity of at least one enzyme, such as β-glucanase, xylanase, amylase, naturally occurring enzymes, and/or protease activity, which is greater than the enzymatic activity which is obtained by holding the wetted cereal without activated spores, or even with the bacteria or moulds from which the spores come.

[0024] In an important aspect, the cereal, activated spores are combined before or after the time of wetting the cereal and the combination is held at a temperature of at least about 5° C and not more than about 30° C, preferably between about 10° to about 20° C and the activated spores are at a concentration in the combination to obtain an increase in enzymatic activity of the malted cereal. In another important aspect, the wetted or moistened cereal and activated spore combination is held for a time and temperature until the cereal has a moisture content of at least about 20 weight percent. In yet another aspect, after the wetted cereal has attained an increased moisture content and has started to germinate, it is dried to a moisture content of not more than about 15 weight percent. In still another important aspect, the moistened cereal and activated spores are held together until the cereal has a moisture content of between about 20 to about 60 weight percent, preferably from about 38 to about 47 weight percent, and has germinated for about 2 to about 7 days, preferably about 3 to about 6 days, at a temperature of from about 10° to about 30° C, preferably from about 14° to about 18° C. In important aspect, the germinated cereal is dried at a temperature of from about 40° to about 150° C, preferably between about 45° and 85° C until the dried malted cereal has a moisture content of from about 2 to about 15 weight percent moisture, preferably from about 4 to abut 7 weight percent moisture. Importantly, the process of the invention may increase β-glucanase activity of a malted cereal by a factor of at least about 4 as compared to a malted cereal prepared without activated spores according to the invention.

[0025] In another important aspect, the present invention provides a malted cereal having a higher quantity of acrospire lengths that were significantly longer in comparison to acrospire lengths when traditional malting methods were used.

[0026] In an important aspect, the cereals which may be used in the invention include barley, wheat and sorghum. In one aspect of the invention the spores of moulds are used. In another aspect, the spores of bacteria are used.

[0027] In another aspect, the cereals may be disinfected or may not be disinfected.

[0028] Preferably, for the preparation of malted barley, the spores from bacteria are from gram positive bacteria or gram negative bacteria, selected from the group of Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp. preferentially Pediococcus halophilus, Pediococcus cerevisiae, Pediococcus damnosus, Pediococcccus hemophilus, Pediococccus parvulus, Pediococcus soyae, Lactococcus spp., Lactobacillus spp. preferentially Lactobacillus acidophilus, Lactobacillus amylovorus, preferentially Lactobacillus amylovorus strain ATCC 33620, Lactobacillus bavaricus, Lactobacillus bifermentans, Lactobacillus brevis var lindneri, Lactobacillus casei var casei, Lactobacillus delbrueckii, Lactobacillus delbrueckii var lactis, Lactobacillus delbrueckii var bulgaricus, Lactobacillus fermenti, Lactobacillus gasserii, Lactobacillus helveticus, Lactobacillus hilgardii, Lactobacillus renterii, Lactobacillus sake, Lactobacillus sativorius, Lactobacillus cremoris, Lactobacillus kefir, Lactobacillus pentoceticus, Lactobacillus cellobiosus, Lactobacillus bruxellensis, Lactobacillus buchnerii, Lactobacillus coryneformis, Lactobacillus confusus, Lactobacillus florentinus, Lactobacillus viridescens, Corynebactrium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., preferentially Bacillus subtilis stain ATCC 6051, preferentially Bacillus circulans, Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp., preferentially Pseudomonas amylophilia, Pseudomonas aeruginosa, Pseudomonas cocovenenans, Pseudomonas mexicana, Pseudomonas pseudomallei, Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., and Proteus spp.

[0029] Preferably, for the preparation of malted barley spores are from fungi which are selected from the group (genera as described by Ainsworth and Bisby's dictionary of the fungi, 8th edition, 1995, edited by DL Hawkworth, PM Kirk, BC Sutton, and DN Pegler (632 pp) Cab International) of Ascomycota preferentially Dothideales, preferentially Mycosphaerellaceae

preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp., Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp., Hypocreales preferentially Hypocreceae preferentially Hypocrea spp., Saccharomycetales preferentially Dipodascaceae, Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnkowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., preferentially Pichia anomala, preferentially Pichia anomala strain ATCC 8168, Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae preferentially Hanseniaspora spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp., Sordariales preferentially Chaetomiaceae, Chaetomium spp., preferentially Chaetomium virescens strain ATCC 32319, Sordariacea preferentially Neurospora spp., Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp. preferentially Mucor circinelloides, Mucor grisecyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Muoor rouxil, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp. preferentially Rhizopus arrhizus, Rhizopus oligosporus, Rhizopus oryzae preferentially Rhizopus oryzae strain ATCC 4858, Rhizopus oryzae strain ATCC 9363, Rhizopus oryzae strain NRRL 1891, Rhizopus oryze strain NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, preferentially Rhizopus thailandensis strain ATCC 20344, Rhizopus formosaensis, preferentially Rhizopus formosaensis strain ATCC 26612, Rhizopus chinensis, Rhizopus cohnii, Rhimpus japonicus, Rhizopus nodosus, Rhizopus delemar, Rhizopus acetorinus, Rhizopus chlamydosporus, Rhizopus circinans, Rhizopus javanicus, Rhizopus peka, Rhizopus salto, Rhizopustritici, Rhizopus niveus, Rhizopus microsporus, Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospom spp., Epicoccum spp., Monilia spp. preferentially Monilia candida, Monilia sitophila, Mycoderma spp., Candida spp., preferentially Candida diddensiae, Candida edax, Candide etchellsii, Candida kefir, Candida krisei, Candida lactose, Candida lambica, Candida melinii, Candida utilis, Candida milleri, Candida mycoderma, Candida parapsilosis, Candida obtux, Candida tropicalis, Candida valida, Candida versatilis,

Candida guilliermondii, Rhodotorula spp., Torulopsis spp., Geotrichum spp. preferentially Geotrichum amycelium, Geotrichum armillariae, Geotrichum asteroides, Geotrichum bipunctatum, Geotrichum dulcitum, Geotrichum eriense, Geotrichum fici, Geotrichum flavo-brunneum, Geotrichum fragrans, Geotrichum gracile, Geotrichum heritum, Geotrichum kiebaknii, Geotrichum penicillatum, Geotrichum hirtum, Geotrichum pseudocandidum, Geotrichum rectangulatum, Geotrichum suaveolens, Geotrichum vanryiae, Geotrichum loubieri, Geotrichum microsporum, Ciadosporium spp., Trichoderma spp. preferentially Trichoderma hamatum, Trichoderma harzianum, Trichoderma koningii, Trichoderma pseudokoningli, Trichodermareesei, preferentially Trichoderma reesei strain ATCC 5875, Trichoderma virgatum, Trichoderma viride, Oldium spp., Alternaria spp. preferentially Alternaria alternata, Alternaria tenuis, Helminthosporium spp. preferentially Helminthosporium gramineum, Helminthosporium sativum, Helminthosporium teres, Aspergillus spp. preferentially Aspergillus ochraseus Group, Aspergillus nidulans Group, Aspergillus versicolor Group, Aspergillus wentii Group, Aspergillus candidus Group, Aspergillus flavus Group, Aspergillus niger Group, Aspergillus oryzae strain ATCC 14156, Penicillum spp. preferentially Penicillum aculeatum, Penicillum citrinum, Penicillum claviforme, Penicillum funiculosum, Penicillum italicum, Penicillum lanoso-viride, Penicillum emersonil, Peniclilum lilacinum, Penicillum expansum, and mixtures thereof.

[0030] Preferably, for the preparation of malted cereals other then malted barley, especially for the preparation of malted wheat, rye, corn, oats, rice, millet, triticale, and sorghum, said bacteria are gram positive or gram negative bacteria selected from the group of Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp., Lactococcus spp., Lactobacillus spp., Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp., Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp. or a mixture thereof; and said fungi are fungi selected from the group of: Ascomycota preferentially Dothideales preferentially Mycophaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp., Hypocreales preferentially Hypocreaceae preferentially Hypocrea spp., Saccharomycetales preferentially Dipodascaceae preferentially

Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccaromycodaceae preferentially Hanseniaspora spp., Schizosaccheromycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp., Zygomycota preferentially Mucoraies preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Clamydomucor spp., Mucor spp., Rhizopus spp.; preferentially Rhizopus oryza strain ATCC 9363, Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cerocospora spp., Epicoccum spp., Monilia spp., Mycoderma spp., Candida spp., Rhodotorula spp., Torulopsis spp., Geotrichum spp., Cladosporium spp., Trichoderma spp., Oldium spp., Alternaria spp., Helminthosporium spp., Aspergillus spp., Penicillium spp.

DEFINITIONS

[0031] As used herein the term "spore" refers to a dormant and highly resistant reproductive cell formed by bacteria and fungi in response to environment conditions that do not favor the growth of the organism. When exposed to favorable environmental conditions, spores are capable of developing into a viable adult organism without fusion with another cell.

[0032] As used herein the term "activated spore" means a spore having one of the following properties:

[0033] i) The spore is swollen such that its size is increased by a factor of between about 1.2 and about 10 over its dormant size; and/or

[0034] ii) one or more germ tubes per spore is formed.

[0035] Activated spores are prepared by one or a combination of the following treatments.

[0036] i) cycles of wetting and/or drying;

[0037] ii) addition of appropriate nutritional supplies (such as a nitrogen source, preferably amino acids and/or a carbon source, preferably mono- or disaccharides) or spore elements;

[0038] iii) exposure to temperature changes, preferably within a temperature range of about 0 to about 80° C;

[0039] iv) exposure to changes in pH, preferably within a pH range of about 2.0 to about 8.0, more preferably about 3.0 to about 6.0.

[0040] The term "germination" as used herein means the beginning or resumption of growth by a seed. In accordance with the process of the present invention, germination begins to occur during and/or after the cereal has been steeped. Germination of cereals is generally understood to mean hydration of the seed, swelling of the cereal and inducing growth of the embryo. Environmental factors affecting germination include moisture, temperature and oxygen level. A rapid increase in cells of the root stem leads to root development, while corresponding growth sends forth a shoot.

[0041] As used herein, the term "steeping" refers to wetting of the cereal. Wetting may include one or more stages over a time and temperature effective for providing a moisture content of between about 20% and about 60% by weight.

[0042] The term "specific activity" as used herein refers to the concentration and activity of an enzyme in a preparation. The specific activity of a preparation is reported as units/mg protein. One unit of enzyme is that amount that catalyzes the formation of 1 mmole of product per minute under defined conditions. The amount of enzyme present in a preparation is measured using standard protein assay techniques and catalytic activity is determined by following the formation of product or removal of substrate over time.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0043] According to a preferred embodiment, the preparation process of malted cereals according to the invention comprises the following steps: a steeping step includes one or more wetting stages or the total time of submersion in water during steeping for physiological reasons does not exceed 30 hours (preferably 10 to 25 hours) or the kilning step includes more then two temperature steps and the microbial cultures which are added, are preferably selected from the group consisting of Rhizopus spp., preferably Rhizopus oryzae, such as Rhizopus oryze strain ATCC9363 and/or Pseudomonas spp., preferably Pseudomonas herbicola.

[0044] According to the invention, the malted cereals are selected from the group of barley, wheat, rye, corn, oats, rice, millet, triticale, sorghum and the like.

[0045] In the process according to the invention, the same or different activated spores are added in one or more time(s). The use of activated spores greatly enhances their contribution to improved malt quality, most likely because of more vigorous growth. The activated spores have one of the following properties: the treated spores are significantly more swollen than their dormant, size, more particularly, the size of the spores is increased by a factor preferably between 1.2 and 10 over their dormant size and/or one or more germ tubes per spore are formed. The activated spores are prepared by subjecting them to environmental changes, preferably by one or a combination of the following treatments;

[0046] (a) cycles of wetting and/or drying;

[0047] (b) addition of appropriate nutritional supplies (such ass a nitrogen source, preferably amino acids and/or a carbon source, preferably mono- or disaccharides) or spore elements;

[0048] (c) exposure to temperature changes, preferably within a temperature range of 0° to 80° C;

[0049] (d) exposure to changes in pH, preferably within a pH range of 2.0 to 8.0, more preferably between 3.0 and 6.0.

[0050] The activated spores may be introduced before or during the malting process. For example, the activated spores may be introduced during the various malting or steeping stages before or after immersion of the cereal.

[0051] The concentration of the spores may vary depending on the conditions of the malting process and the type of active spore being utilized. Generally about 1×10^2 to about 1×10^7, preferably about 1×10^3 to about 1×10^5 activated spores per gram air dry cereal is utilized.

[0052] The present invention also concerns the malted cereals obtained according to the process of the invention, which present improved European Brewery Convention analysis results. Said improvements may have to do with modification and/or increased hydrolytic enzyme activities. At the same time, a decreased level of toxins, an increased microbial safety by e.g., outcompeting undesirable microbial flora such as Fusarium and/or an increased acceptability compared to the malted cereals according to the state of the art, may be observed.

[0053] For instance, the malted cereals according to the invention may have a lower β-glucan content or a higher enzyme activity such as, for example, β-glucanase or xylanase activity (as represented in the following examples and figures) than the malted cereals according to the state of the art. This allows for a better processability of the malt in wort and beer production as exemplified by increased rates of filtration.

[0054] The activated spores and cereal may be combined and wetted by submersion in water to steep the combination which should not exceed 30 hours. The activated spores can also be sprayed on the barley during the steep period or during the germination process. The pH during this period should be from about 1.5 to about 14, preferably about 4 to about 6. β-glucanase activity of malted barley made according to the invention is higher than 700 units/kg and xylanase activity is higher than 250 units/kg.

[0055] An object of the present invention concerns the use of the malted cereals according to the invention for the preparation of beverages.

[0056] The invention is also related to these improved beverages.

[0057] The improved malted cereals according to the invention could also be used in other biotechnological processes well known by the Person Skilled in the Art, in which in most cases advantage is taken of their improved quality.

[0058] The present invention will be further described in various examples in view of the following drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] FIG. 1 represents the β-glucanase activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1).

[0060] FIG. 2 represents the xylanese activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1).

[0061] FIG. 3 represents the β-glucanase activity of malted barley obtained according to the preparation process of example 3. (legend: see example 3).

[0062] FIG. 4 represents the xylanase activity of malted barley obtained according to the preparation process of example 3. (legend: see example 3).

[0063] FIG. 5 represents the relative increase factory (R.I.F.) for bacterial populations (see text, malt evaluation, example 2) (legend: see example 2).

EXAMPLE 1

[0064] **1. Preparation of Microbial Cultures**

[0065] Strain

[0066] S46: Rhizopus oryzae ATCC 9383.

[0067] Preparation of the Spore Suspension

[0068] the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 10 days at 28° C;

[0069] the spores were harvested by flooding the cultures with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;

[0070] the spore suspension was washed twice with sterile physiological saline (0.9% NaCl) by centrifugation (5500 rpm, Sorvall type SS-34.RTM., for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl);

[0071] the spore density was determined microscopically using a Thoma counting chamber.

[0072] Activation of the Spore Suspension

[0073] 10^7 spores were transferred into 20 ml of sterile, acidifed TSB (Tryptic Soy Broth, Oxoid), pH=4.0 and incubated in a shaking water bath during 5 to 6 hours at ±42° C;

[0074] The activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min.), washed once with sterile physiological saline (0.9% NaCl) by centrifugation (3500 rpm, Sorvall type SS-34.RTM., for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

[0075] 2. Barley

[0076] Plaisant - 1994 French harvest.

[0077] 3. Process.

[0078] Setup

[0079] Malts were made by four different malting processes:

[0080] A1. traditional malting

[0081] (without inoculation of any spore suspension)

[0082] B1. malting process using non-activated spores

[0083] (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)

[0084] C1. malting process according to the invention

[0085] (inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

[0086] D1. malting process according to the invention

[0087] (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Rhizopus oryzme ATCC 9363)

[0088] Steeping

[0089] the steeping was carried out on a 2 kg base with a total water (tap water) to air dry barley ratio of 1.5:1;

[0090] use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which perforated plates were placed;

[0091] temperature was only controlled during the wet stages, during the air rest stages, the system was allowed to reach room temperature (±20° C);

[0092] during the whole steeping period, the barley was aerated (4 liter sterile air per minute);

[0093] steeping was carried out by immersion using the following scheme;

	Temperature (°C)	**Duration (h)**
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

[0094] Addition of the Microbial Cultures

[0095] ± 480 g of steeped barley was immersed in 0.5 liter of tap water which contained no spores (A1), non-activated spores of Rhizopus oryzae ATCC 9363 (B1) or activated spores of Rhizopus oryzae ATCC 9363 (C1, according to the invention); for B1 and C1, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;

[0096] during the steeping, 10^4 activated spores per gram air dry barley were inoculated to the water of the first wet stage (D1);

[0097] the fluid was removed by draining.

[0098] Germination

[0099] germination was carried out in a cylindrical container with perforated lids at a temperature of 16°-18° C during 4 days;

[0100] air was supplied by natural diffusion;

[0101] the containers were slowly rotated on an electrically controlled roller system (Cellroll®, Tecnorama); i.e., every two hours the containers were rolled for 15 min. at 1 rpm.

[0102] Kilning

[0103] the kilning was carried out in a Joe White malting unit (Australia),

	Air flow (%)	**Recir. Air (%)**	**Temp. (°C)**	**Durat. (h)**
First kilning stage	25	0	62	3:00
Second kilning stage	25	0	65	2:00
Third kilning stage	25	0	68	2:00
Fourth kilning stage	25	25	73	2:00
Fifth kilning stage	25	50	78	1:00
Sixth kilning stage	25	75	80	2:00
Seventh kilning stage	25	100	83	6:00
Shut down air off				Time out

[0104] 4. Methods of Analysis and Results

[0105] Methods for determination and units of moisture, extract, extract difference, color, total protein content, soluble protein content, Kolbach index, pH, diastatic power, according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

[0106] Methods for determination and units of turbidity, friability, homogeneity, whole grains, b-glucan content, according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau, supplement published in 1989).

[0107] Postcoloration of the wort is determined after boiling the congress wort under reflux at 108° C during 2 hours.

[0108] The viscosity of the congress wort is determined with the Delta-viscosimater.

[0109] For the determination of the filtration volume, the congress wort is filtered over a Schleicher and Schuell 597 ½ folded filter. The volume (in mil) that is obtained after 1 hour of filtration is the filtration volume of the wort.

[0110] Modification is determined with the Calcofluor apparatus (Haffmans) according to the Carisberg method (Analytica-Europeon Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

[0111] The β-glucanase and xylanase activities are determined with the β-glucazym method (Megazme (Austr.) Pty Ltd. (April, 1993) and the xylazym method ((Megazyme (Austr.) Pty Ltd. (September 1995)), respectively.

	Traditional Malting Process (A1)	**Malting Process using non-activated spores (B1)**	**Malting Process according to the Invention (C1)**	**Malting Process according to the Invention (D1)**
Moisture	3.9	4.1	3.8	4.3
Extract	80.3	80.4	80.3	79.8
Extract Difference	0.8	0.8	0.4	1.1
Color	3.3	3.3	4.1	4.1
Wort Turbidity	1.3	1.2	0.7	0.8
Postcoloration	6	6	7.3	7.5
Total Protein Content	10.1	10.3	10	10.1
Soluble Protein	4.1	4.4	4.8	5.2

	Traditional Malting Process (A1)	**Malting Process using non-activated spores (B1)**	**Malting Process according to the Invention (C1)**	**Malting Process according to the Invention (D1)**
Content				
Kolbach Index	40.6	42.7	48	51.0
Viscosity	1.57	1.52	1.52	1.54
pH	6.05	6.3	5.87	5.79
Diastatic Power	345	349	352	419
Whole Grains	0.3	0.3	0.1	ND
Friability	83	82	83.9	ND
Homogeneity	98.5	97.9	98.6	ND
β-glucan content	122	108	46	<40
Filtration Volume	210	265	290	275
Modification	88.2	90.5	93.4	ND
β-glucanase Activity	214	371	683	3856
Xylanase Activity	28	34	56	984

[0112] FIGS. 1 and 2 represent the β-glucanase and xylanase activity, respectively of the obtained malted barley (A1, B1, C1, D1). These malted barleys are obtained according to a traditional malting process (A1) or using non-activated spores during the malting process (B1) or according to the above-described malting process of the invention (C1, D1). The β-glucanase activity was determined with the β-glucazym method (Megazyme (Austr.) Pty Ltd. (April, 1993)). Therefore, malt β-glucanase activity (U/kg) was calculated at 380 x E (590 nm)+20. The xylanase activity was determined with the endo 1-4-xylazym method (Megazyme (Austr.) Pty Ltd. (September 1995)). Therefore, malt xylanese activity (U/kg) was calculated as (46.8 x E (590 nm)+0.9)x 5).

EXAMPLE 2

[0113] <u>1. Preparation of Microbial Cultures</u>

[0114] <u>Strain</u>

[0115] S46: Rhizopus oryzae ATCC 9363.

[0116] as described in Example 1.

[0117] <u>Preparation of the Spore Suspension</u>

[0118] as described in Example 1.

[0119] <u>Activation of the Spore Suspension</u>

[0120] as described in Example 1.

[0121] <u>2. Barley</u>

[0122] Stander - 1995 North American harvest.

[0123] <u>3. Process</u>

[0124] <u>Setup</u>

[0125] Malts were made by six different malting processes:

[0126] A2, traditional malting process

[0127] (without inoculation of any spore suspension)

[0128] B2, malting process using non-activated spores

[0129] (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)

[0130] C2, malting process according to the invention

[0131] (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9383)

[0132] D2, malting process according to the invention

[0133] (inoculation of the steeped barley during the second wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

[0134] E2, malting process according to the invention

[0135] (inoculation of the steeped barley during the third wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

[0136] F2. malting process according to the invention

[0137] (inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

[0138] <u>Steeping and Addition of the Microbial Cultures</u>

[0139] the steeping was carried out on a 300 g base with a total water (tap water) to air dry barley ratio of 5:3;

[0140] use was made of 2000 ml flasks;

[0141] a temperature of 18° C. was maintained during the wet stages and during the air rest stages;

[0142] during the whole steeping period, the barley was aerated by means of compressed air;

[0143] steeping was carried out by immersion using the following schedule;

	Duration (h)
First wet stage	6:00
First air rest stage	18:00
Second wet stage	5:00
Second air rest stage	19:00
Third wet stage	2:00

[0144] during the steeping, 10^4 activated spores per gram of air dry barley were inoculated to the water of the first wet stage (C2), of the second wet stage (D2) or of the third wet stage (E2) before immersion of the barley;

[0145] the steeped barley was immersed in 0.5 liters of tap water which contained no spores (A2), non-activated (B2) or activated (C2, D2, E2, F2) spores;

[0146] for B2, and F2, the steeped barley was inoculated with 10^4 spores per gram of air dry barley,

[0147] the fluid was removed by draining.

[0148] Germination

[0149] as described in Example 1.

[0150] Kilning

[0151] as described in Example 1.

[0152] Malt evaluation

[0153] Determination of the Increase of the Bacterial Population

[0154] To judge the evolution of the bacterial population during the malting process, a relative increase factor (R.I.F.) was determined by dividing the total bacterial count occurring on the green malt by the total bacterial count occurring on the barley. The total bacterial count was determined after plating appropriate dilutions of an extract of the kernels on Tryptic Soy Agar (Oxoid) supplemented with 100 ppm pimaricine and after incubation at 28° C for 3 days.

[0155] FIG. 5 shows the increase of the bacterial population during the malting according to the preparation process of Example 2.

EXAMPLE 3

[0156] 1. Preparation of Microbial Cultures

[0157] Strain

[0158] S 46: Rhizopus oryzae ATCC 9363

[0159] Preparation of the Spore Suspension

[0160] as described in Example 1

[0161] Activation of the Spore Suspension

[0162] as described in Example 1

[0163] 2. Barley

[0164] Plaisant - 1994 French harvest;

[0165] 3. Process

[0166] Setup

[0167] Malts were made by three different malting processs:

[0168] A3. traditional malting

[0169] (without inoculation of any spore suspension)

[0170] B3. malting process using non-activated spores

[0171] (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)

[0172] C3. malting process according to the invention

[0173] (inoculation of the steeped barley with a suspension of activted spores of Rhizopus oryzsa ATCC 9363)

[0174] Steeping

[0175] the steeping was carried out on a 2 kg base air dry barley with a total water (tap water) to air dry barley ratio of 1.5:1;

[0176] the pH of the steeping water was controlled at pH=5.5 by addition of lactic acid and NaOH;

[0177] a fermentor (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed, was used for steeping;

[0178] temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (ca. 20° C);

[0179] during the whole steeping period the barley was aerated (4 liters startle air per minute);

[0180] steeping was carried out by immersion using the following schedule:

	Temperature (°C)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

[0181] Addition of the Microbial Cultures

[0182] 460 g of steeped barley was immersed in 0.5 liters of tap water which contained no spores (A3), non-activated spores of Rhizopus oryzae ATCC 9363 (B3) or activated spores of Rhizopus oryzae ATCC 9363 (C3 according to the invention); for B3 and C3, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;

[0183] the fluid was removed by draining.

[0184] Germination

[0185] as described in Example 1

[0186] Kilning

[0187] as described in Example 1

[0188] 4. Methods of analysis and results

[0189] These were as described in Example 1 (4. Methods of Analysis and Results).

[0190] See table on next page. In this table:

[0191] A1/3: Traditional malting process

[0192] B1/3: Malting process using non-activated spores

[0193] C1/3: Malting process according to the invention

	Example 3 pH control of the steeping water (pH – 5.5)			**Example 1 No pH control of the steepingwater**		
	A3	B3	C3	A1	B1	C1
Moisture	3.8	3.6	3.7	3.9	4.1	3.8
Extract	78.9	80.2	80.7	80.3	80.4	80.3
Extract Difference	0.6	0.7	0.4	0.8	0.8	0.4
Color	3.2	4.2	4.4	3.3	3.3	4.1
Wort Turbidity	1	1	0.8	1.3	1.2	0.7
Postcoloration	5.1	7	7.2	6	6	7.3
Total Protein Content	10.2	10.1	10	10.1	10.3	10
Soluble Protein Content	4	4.4	4.8	4.1	4.4	4.8
Kolbach Index	39.2	43.6	48	40.6	42.7	48
Viscosity	1.52	1.53	1.52	1.57	1.52	1.52
pH	6.02	5.97	5.91	6.05	6.03	5.87
Diastatic Power	348	333	355	345	349	352
Whole Grains	0.2	0.2	0.1	0.3	0.3	0.1
Friability	81	81	85	83	82	83.9
Homogeneity	97.6	97.8	98.9	98.5	97.9	98.6
β-glucan content	190	57	40	122	108	46
Filtration Volume	210	215	200	210	265	290
Modification	84.1	85.5	87.4	88.2	90.5	93.4
β-glucanase Activity	202	931	1322	214	371	683
Xylananse Activity	43	65	71	28	34	56

[0194] FIG. 3 represents the β-glucanase activity, measured according to β-Glucazym method [Megazyme (AUSTR) Pty. Ltd.] of the malted cereals A3, B3 and C3. Malt .beta.-glucanase activity (U/kg) was calculated as described in example 1. A3 was obtained by the traditional malting process with pH control of the steeping water (pH=5.5). B3 resulted from the malting process according to the invention with the inoculation of steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363 and with pH control of the steeping water (pH=5.6). C3 was obtained by the malting process according to the invention with the inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363 and with pH control of the steeping water (pH=5.5).

[0195] These results show the increased β-glucanase activity when the pH of the steeping water is maintained at around 5.5.

[0196] FIG. 4 gives the corresponding results for xylanase activity. These were measured according to xylazym method, Megazyme ((AUSTR) Pty. Ltd. (September 1995)). Malt xylanase activity was calculated as described in Example 1.

[0197] Comparison of the β-glucanase activity obtained according to examples 1 and 3 with the β-glucanase activity according to the state of the art as described in WO94/29430.

[0198] In order to compare the improved results regarding β-glucanase activity by the present invention, we defined the factor m as follows:

$$m = \frac{\beta\text{-glucanase activity of the treated malt}}{\beta\text{-glucanase activity of the controlled malt}}$$

[0199] This factor was calculated for control malt and malted treated with Rhizopus oryzae ATCC 9363 as described in Examples 1 and 3 of the present invention.

[0200] It was also calculated for the data described in WO94/29430 (Example 1) where

Geotrichum candidum was used.

[0201] Both as described in WO94/29430, and in the present application, β-glucanase activity was determined with the beta-glucazyme method [Megazyme (AUSTR) Pty. Ltd. (April 1993)]. Therefore, malt β-glucanase activity (U/kg) was calculated as 380 x E(590nm)+20 and one unit of activity was defined as the amount of enzyme required to release one micromole of reducing sugar equivalents per minute under the defined above conditions.

[0202] Comparison of the Results:

State of the Art				**Invention**			
	m		m	Ex. 1	m	Ex. 3	m
Gc*	1.48	Gc*	1.98	B1/A1	1.73	B3/A3	4.61
				C1/A1	3.19	C3/A3	6.54
				D1/A1	18.02		

*Gc: Geotrichum candidum

[0203] The results clearly show that the present invention provides for a more drastic increase in malt β-glucanase activity than that described earlier (WO 94/29430).

[0204] It thus appears that it is possible to obtain malted cereals having a β-glucanase activity increased by at least a factor 4 compared to the conventional malting process wherein the addition of microbial culture is omitted.

[0205] From FIGS. 2 and 4, it also appears that it is possible to obtain malted cereals having a xylanase activity increased by at least a factor 4 compared to conventional malting process wherein the addition of microbial culture is omitted.

EXAMPLE 4

[0206] 1. Preparation of the microbial cultures

[0207] Strain

[0208] S40: Aspergillus oryzae ATCC 14156

[0209] Preparation of the spore suspension

[0210] the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 7 days at 281° C.;

[0211] the spores were harvested by flooding the culture with sterile physiological saline (0.9%/NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;

[0212] the spore suspension was washed once with sterile physiological saline (0.9% NaCl) by centrifugation (5500 rpm. Sorval type SS-34.RTM., for 15 min) and resuspended in sterile physiological saline (0.9% NaCl);

[0213] the spore density was determined microscopically using a Thoma counting chamber.

[0214] Activation of the Spore Suspension

[0215] 5 x 10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH=5.0 and incubated in a shaking water bath during 3 hours (1) or 1 hour (2) at 35° C,

[0216] 2. Cereal

[0217] Clarine barley - 1995 French harvest

[0218] 3. Process

[0219] Malts were made by two different malting process:

[0220] A4. traditional malting

[0221] (without inoculation of any spore suspension)

[0222] E4. malting process according to the invention

[0223] (inoculation of the steeped barley during the first and third wet stage with a suspension of activated spores of Aspergillus oryzae ATCC 14156)

[0224] Steeping

[0225] as described in Example 1

[0226] Addition of the microbial cultures

[0227] during the steeping, 5 x 10^3 activated spores (1) per gram air dry barley were inoculated to the water of the first wet stage and 10^4 activated spores (2) per gram air dry barley were inoculated to the water of the third wet stage (E4);

[0228] Germination

[0229] germination of ± 460 g steeped barley was carried out in cylindrical containers with perforated lids at a temperature of 16°-18° C during 4 days;

[0230] air was supplied by natural diffusion;

[0231] the containers were slowly rotated on an electronically controlled roller system (Cellroll®, Tecnorama); i.e., every two hours the containers were rolled for 15 min at 1 rpm.

[0232] Kilning

[0233] as described in Example 1

[0234] 4. Methods of the analysis and results

[0235] These were described in Example 1 (4, Methods of Analysis and Results) Method for the determination of the acrospire length according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brsuerel und Getränke-Rundschau).

		0	**0-¼**	**¼-½**	**½-¾**	**¾-1**	**>1**
1 day germination	A4	0	1	60	39	0	0
1 day germination	E4	0	0	11	77	12	0
4 days germination	A4	1	1	31	64	3	0
4 days germination	E4	1	0	1	42	49	7

[0236]

	Traditional Malting Process (A4)	**Malting Process According to the Invention (E4)**
Moisture	4.3	4.0
Extract	80.8	81.1
Extract Difference	1.0	0.3
Color	2.8	3.2
Wort Turbidity	1.6	1.0
Postcoloration	4.8	5.4
Total Protein Content	10.1	10.0
Soluble Protein Content	3.9	4.5
Kolbach Index	38.6	44.7
Viscosity	1.57	1.48
pH	5.98	5.89
Diastatic Power	197	201
Whole Grains	1.3	0.6
Friability	81	89
Homogeneity	95.0	98.4
β-glucan content	378	132
Filtration Volume	300	310
Modification	83.9	89.8
β -glucanase Activity	309	392
Xylananse Activity	27.82	17.62

EXAMPLE 5

[0237] 1. Preparation of the Microbial Cultures

[0238] Strains

[0239] S40: Aspergillus oryzae ATCC 14156

[0240] S46: Rhizopus oryzae ATCC 9363

[0241] Preparation of the Spore Suspensions

[0242] As described in Example 4

[0243] Activation of the Spore Suspensions

[0244] S40:

[0245] 5 x 10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid) pH=5.0 and incubated in a shaking water during 1 hour at 35° C.;

[0246] the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS34®, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

[0247] S45:

[0248] 5 x 10^7 spores were transferred into 20 ml of sterile, activated TSB (Tryptic Soy Broth, Oxoid) pH=4.0 and incubated in a shaking water bath during 5 hours at 42° C.;

[0249] the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS34®, for

15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

[0250] 2. Cereal

[0251] Clarine - 1995 French harvest

[0252] 3. Process

[0253] Setup

[0254] Malts were made by two different malting processes:

[0255] A5. traditional malting

[0256] (without inoculation of any spore suspension)

[0257] F5. malting process according to the invention

[0258] (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Aspergillus oryzae ATCC14156 and after steeping with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

[0259] Steeping

[0260] As described in Example 1

[0261] Addition of the Microbial Cultures

[0262] during steeping, 10^4 activated spores of Aspergillus oryzae ATCC 14156 per gram air dry barley were inoculated to the water of the first wet stage (F5, according to the invention);

[0263] ± 460 g of steeped barley was immersed in 0.5 liters of tap water which contained no spores (A5) or activated spores of Rhizopus oryzae ATCC 9363 (F5, according to the invention); for F5 the steeped barley was inoculated with 10^4 activated spores per gram air dry barley;

[0264] the fluid was removed by draining.

[0265] Germination

[0266] As described in Example 4.

[0267] Kilning

[0268] As described in Example 1.

[0269] 4. Methods of Analysis and Results

[0270] These were as described in Example 1 (4. Methods of Analysis and Results).

[0271] Method for the determination of the acrospire length according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

		0	**0-¼**	**¼-½**	**½-¾**	**¾-1**	**>1**
1 day germination	A5	1	1	53	44	1	0
1 day germination	F5	0	1	21	73	5	0
4 days germination	A5	0	0	0	29	63	8
4 days germination	F5	0	0	0	13	63	24

[0272] It was noted that the use of activated spores of Aspergillus oryzae ATCC improved the malt analytical specifications.

[0273] Furthermore, it was found that during the malting process, the barley acrospire lengths were significantly longer using the process according to the invention in comparison to the

traditional malting process.

	Traditional Malting Process (A5)	Malting Process According to the Invention (F5)
Moisture	3.9	4.2
Extract	81.4	81.8
Extract Difference	0.9	1.1
Color	3.8	3.8
Wort Turbidity	1.4	1.0
Postcoloration	6.9	6.4
Total Protein Content	10.1	10.2
Soluble Protein Content	4.8	5.2
Kolbach Index	48.0	51.3
Viscosity	1.51	1.50
pH	5.88	5.82
Diastatic Power	199	214
Whole Grains	0.8	1.1
Friability	89	95
Homogeneity	98.3	98.3
β-glucan content	120	51
Filtration Volume	270	220
Modification	96.8	98.6
β-glucanase Activity	263	907
Xylananse Activity	28.86	57.76

EXAMPLE 6

[0274] 1. Preparation of the Microbial Cultures

[0275] Strains

[0276] S46: Rhizopus oryzae ATCC 9363

[0277] Preparation of the Spore Suspensions

[0278] As described in Example 4

[0279] Activation of the Spore Suspensions

[0280] 5 x 10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid) pH=4.0 and incubated in a shaking water both during 5 hours at 42° C.;

[0281] the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

[0282] 2. Cereal

[0283] Wheat: Mobil - 1996 Belgian harvest

[0284] 3. Process

[0285] Setup

[0286] Malts were made by two different malting processes:

[0287] A6. traditional malting

[0288] (without inoculation of any spore suspension)

[0289] D6. malting process according to the invention

[0290] (inoculation of the steeped wheat during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

[0291] Steeping

[0292] the steeping was carried out in a 2 kg base with a total water (tap water) to air ratio of 1.5:1;

[0293] use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed;

[0294] temperature was only controlled during the wet stages; during the air rest stages, the system was allowed to reach room temperature (±20° C);

[0295] during the whole steeping period the wheat was aerated (4 liter sterile air per minute);

[0296] steeping was carried out by immersion using the following scheme:

	Temperature (°C)	**Duration (h)**
First wet stage	13	6:00
First air rest stage	20	16:00
Second wet stage	14	4:00
Second air rest stage	20	16:00
Third wet stage	16	2:00

[0297] Addition of the Microbial Culture

[0298] during steeping, 10^4 activated spores of per gram air dry wheat were inoculated to the water of the first wet stage (D6);

[0299] Germination

[0300] As described in Example 4.

[0301] Kilning

[0302] As described in Example 1.

[0303] 4. Methods of Analysis and Results

[0304] These were as described in Example 1 (4. Methods of Analysis and Result).

	Traditional Malting Process (A6)	**Malting Process According to the Invention (D6)**
Moisture	5.5	5.4
Extract	83.6	85.5
Extract Difference	1.0	0.6
Color	3.9	7.6
Wort Turbidity	1.4	1.4
Postcoloration	5.8	11.5
Total Protein Content	14.0	14.8
Soluble Protein Content	4.9	9.7
Kolbach Index	35.0	65.5
Viscosity	1.99	1.79
pH	6.02	5.63
Diastatic Power	183	193
Whole Grains	19.4	20.2
Friability	35	42
Homogeneity	79.4	78.7
Filtration Volume	220	295
B-glucanase Activity	10.9	16,640
Xylananse Activity	16.85	1,620.1

EXAMPLE 7

[0305] <u>1. Preparation of the Microbial Cultures</u>

[0306] <u>Strain</u>

[0307] S46: Rhizopus oryze ATCC 9363

[0308] <u>Preparation of the Spore Suspension</u>

[0309] the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 7 days at 28° C.;

[0310] the spores were harvested by flooding the culture with sterile physiological saline (0.9%

NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;

[0311] the spore suspension was washed once with sterile physiological saline (0.9% NaCl) by centrifugation (3500 rpm, Jouan C312, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl);

[0312] the spore density was determined microscopically using a Thoma counting chamber.

[0313] Activation of the Spore Suspension

[0314] 5.times.10.sup.7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid) pH=4.0 and incubated in a shaking water bath during 5 hours at 42° C.

[0315] 2. Cereal

[0316] Sorghum (S14)

[0317] 3. Process

[0318] Setup

[0319] Malts were made by two different malting processes:

[0320] A7. traditional malting

[0321] (without inoculation of any spore suspension)

[0322] D7. malting process according to the invention

[0323] (inoculation of the sorghum during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363).

[0324] Cleaning

[0325] washing of the sorghum is performed by using 6 liters tap water per kilogram sorghum and by removing the excess water.

[0326] Steeping

[0327] the steeping was carried out in a 2 kg base with a total water (top water) to air ratio of 1.5:1;

[0328] use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed;

[0329] temperature was only controlled during the wet stages; during the air rest stages, the system was allowed to reach room temperature (± 20° C);

[0330] during the whole steeping period the barley was aerated (2 liter sterile air per minute);

[0331] steeping was carried out by immersion using the following scheme:

	Temperature (° C)	**Duration (h)**
First wet stage	28	10:00
First air rest stage	20	4:00
Second wet stage	28	10:00
Second air rest stage	20	4:00
Third wet stage	28	10:00
Third air wet stage	20	4:00

[0332] Addition of the Microbial Cultures

[0333] during steeping, 10^4 activated spores (1) per gram air dry sorghum were inoculated to the

water of the first wet stage (D7).

[0334] Germination

[0335] germination of ± 460 g steeped sorghum was carried out in cylindrical container with perforated lids at a temperature of 28° C during 4 days;

[0336] air was supplied by natural diffusion;

[0337] the containers were slowly rotated on an electronically controlled roller system (Cellroll®, Tecnorama); i.e., every two hours the containers were rolled for 15 min. at 1 rpm.

[0338] Kilning

[0339] As described in Example 1.

[0340] 4. Method of Analysis and Results

[0341] These were as described in Example 1 (4. Methods of Analysis and Results).

	Traditional Malting Process (A7)	**Malting Process According to the Invention (D7)**
β-glucanase Activity	98	991
Xylanase Activity	524.72	413.48